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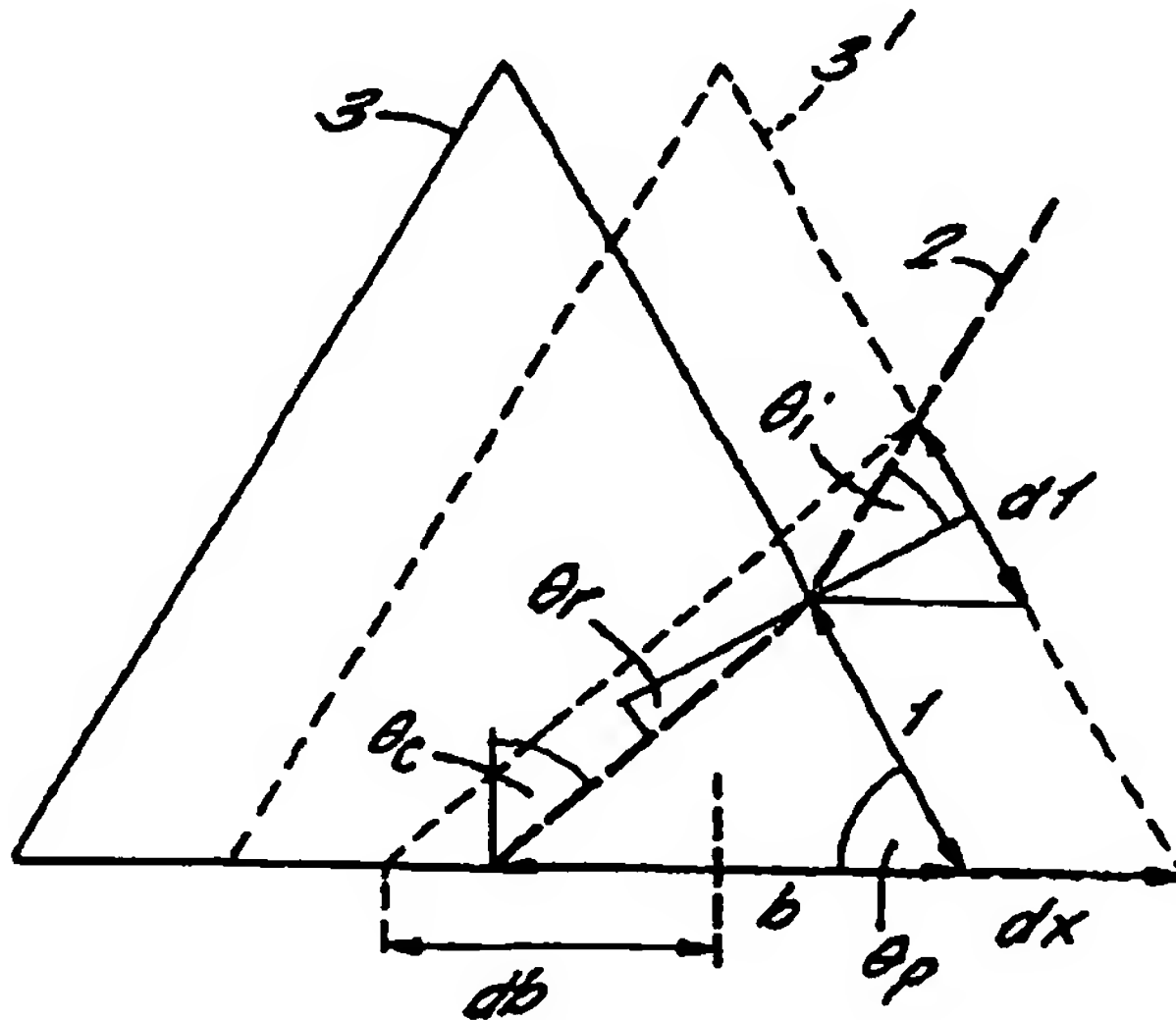
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(57) There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.



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PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS

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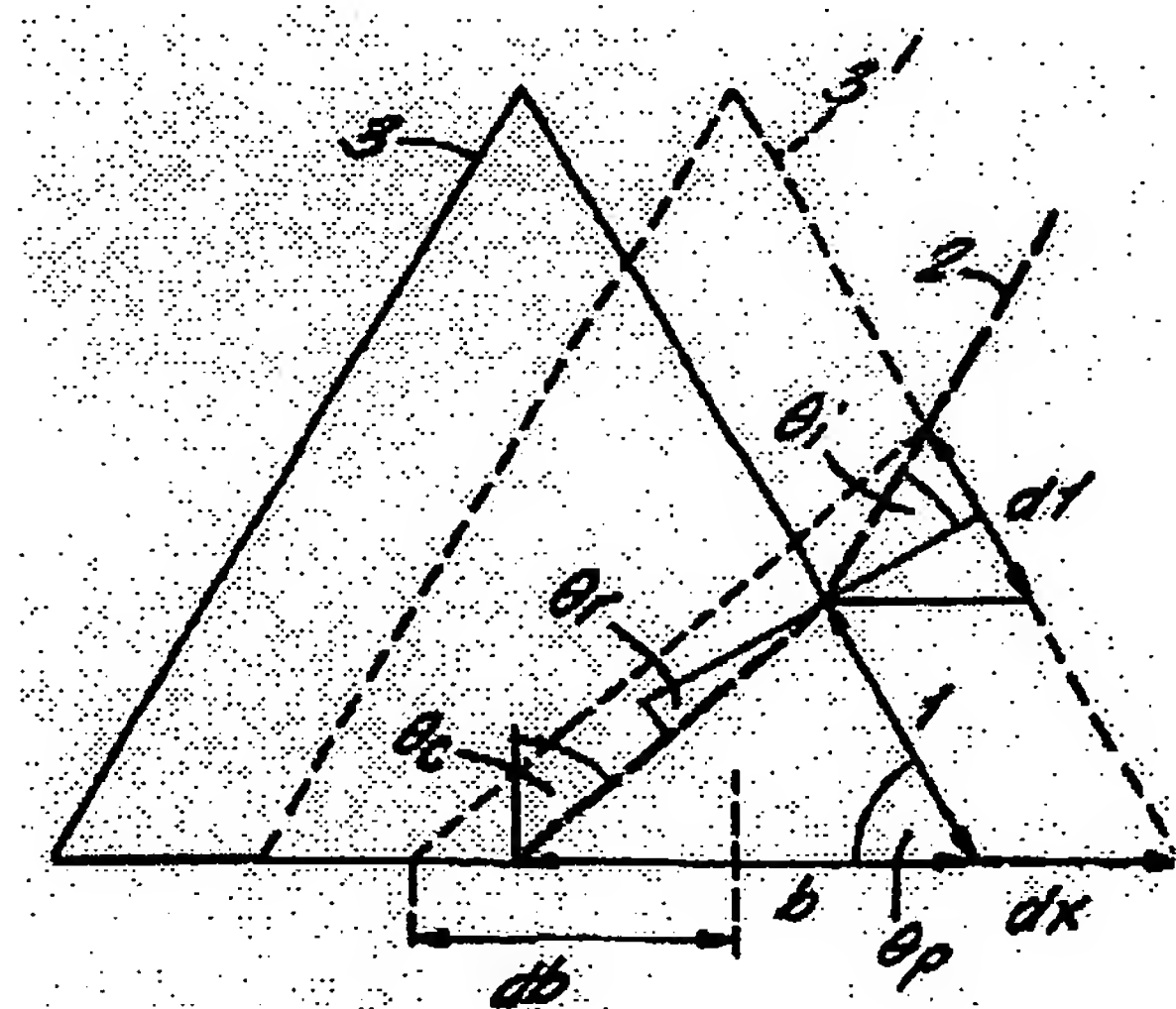
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Abstract of WO03062897

There is disclosed a prism for use in scanning applications such as total internal reflection microscopy in which the prism is translated relative to an incident light beam. A geometry is disclosed which cancels walk of the beam footprint at the base of the prism.



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Description of WO03062897

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PRISM DESIGN FOR OPTICAL SCANNING APPLICATIONS

The present invention relates to scanning applications in which a sample, together with an angular optic a triangular or trapezoidal prism, are scanned in a fixed laboratory frame of reference. The fixed laboratory frame is defined by a fixed light source, generating a fixed light beam and a detector. The angular optic couples the light beam to the base interface of the angular optic where the sample is located, such that the beam is incident on the base interface at an off normal angle. In such applications it is desirable that the intercept of the light beam at the base interface of the angular optic (the "footprint") remains stationary in the laboratory frame as the optic and sample are scanned so that there is no loss of image integrity at the detector.

One example of such an application is Total Internal Reflection Microscopy, which is a technique for observing samples illuminated by an evanescent wave. Total internal reflection occurs when a beam of light travelling through a very dense medium such as glass encounters an interface with a less dense medium such as air or water, at an angle to the normal which is greater than the critical angle for the interface. The critical angle for a glass/water interface is given by Fresnel's Law of Refraction as: $S = \sin(n_{\text{water}}/n_{\text{glass}})$

At angles greater than the critical angle, when total internal reflection takes place, an electric field component of the light penetrates through the interface into the water as an evanescent wave. The evanescent wave has the same wavelength as the incident beam but penetrates only a very short distance into the water, typically no more than 1 μm . The evanescent wave decays exponentially from the interface into the water with a characteristic

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penetration depth dependent.. on the wavelength and angle of incidence of the totally internally reflected light.

In Total Internal Reflection Fluorescence Microscopy, fluorophores may be excited by the light in the evanescent field if they are close to the glass/water interface, but fluorophores further away in the bulk of the solution will not be excited. The result is that images with very low background fluorescence are obtained. Figure 1 shows a typical instrument set up used in Total Internal Reflection Fluorescence Microscopy. A sample is placed such that it is located directly on the interface of the base of a light coupling optic or dispersion prism. Alternatively, a glass slide may be optically matched to the prism, with the sample located on the base of the slide. Total internal reflection then occurs at the base of the slide.

Typically, the objective lens and external light source are fixed in the lab frame and the sample on which the light coupling optic or prism is fixed is scanned in a plane perpendicular to the objective lens axis. The prism therefore moves relative to the objective lens and the light source. Conventionally a 45 or 60 degree dispersion prism is used, but to obtain light beams incident on the base of the prism at angles close to and greater than the critical angle, the light must usually be incident on the input face of the prism at off normal angles of incidence to achieve refraction of the beam at the air/glass interface. The deviation of the beam causes the reflection footprint at the base of the prism to walk as the prism is translated dx towards or away from the light source. In a limiting case light propagating parallel to the prism base will be refracted such that the footprint at the prism base moves equally and in the same direction as the prism ($db/dx=0$).

In this case the footprint moves dx in the lab frame and the illuminated area moves rapidly away from the imaging lens as the sample is scanned.

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It is an object of the present invention to obtain a footprint which is static in the laboratory frame of reference, defined by the objective lens and light source, such that the area illuminated at the glass/aque interface does not move away from the optical axis of the lens as the sample and prism are scanned.

According to the present invention, a scanning apparatus comprises:- a light source for generating a light beam; and an angular prism coupled to a sample at a base interface; characterised in that the base angle of the prism satisfies the equations:- $(\cos^2 \theta_c - \sin^2 \theta_p) (\tan \theta_i \sin \theta_p + \cos \theta_p) = 1 \cos \theta_c \cos (\theta_p \pm \theta_c)$ and $n_i \sin \theta_i = n_p \sin (\theta_p - \theta_c)$ wherein θ_c is the coupling angle required for light incident at the base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

According to the present invention, a scanning method comprises the steps of:- generating a light beam;

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providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and moving the prism and sample relative to the light beam; characterised in that the base angle θ_p of the prism satisfies the equations:- $(\cos^2 \theta_c - \sin^2 \theta_p) (\tan \theta_i \sin \theta_p + \cos \theta_p) = 1 \cos \theta_c \cos (\theta_p \pm \theta_c)$ and $n_i \sin \theta_i = n_p \sin (\theta_p - \theta_c)$ wherein θ_c is the coupling angle required for light incident at the interface the base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

It has been found that, if the prism satisfies the above criteria, a solution exists where the footprint of the light beam on the base of the prism walks in an equal and opposite direction to the prism's translation in the lab frame i. e. $dx = -db$. Therefore, the point where the light incident to the prism intercepts the prism base is fixed in the lab frame. This results in scanning of the sample without movement of the footprint with respect to a detector fixed in the lab frame and hence no loss of image integrity.

A solution is found wherein $\theta_p = \theta_c$ and $F > 0$.

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Preferably, the apparatus comprises a total internal reflection microscopy apparatus and includes means detecting interaction of the sample at the base of the prism or optically matched slide with an evanescent wave formed by total internal reflection of the light beam at the base of the prism or at the base of a slide which is optically matched to the prism. Preferably, the method according to the present invention includes the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

For light entering the prism from air, n_i and therefore $\sin \theta_i = n_p \sin (\theta_p - \theta_c)$

For total internal reflection to occur at the base interface, must be greater than or equal to the critical angle for the interface i.e. $\sin^{-1} (n_s/n_p)$

Wherein n_s is the refractive index of the sample medium.

In total internal reflection microscopy, it is preferable that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium.

It is generally preferable that θ_c is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam it is preferable to have θ_c slightly above the critical angle.

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This also accommodates minor variations in the refractive indices of the interfacial media.

For a quartz/water interface at the base of the prism, where $n_p = 1.46$, the critical angle of the base interface is 66° . A preferred value for θ_c would be 68° . A unique solution is found wherein $e_i = 0$, and $\theta_p = 68^\circ$.

A preferred embodiment of the present invention will now be described with reference to the accompanying drawings; in which:-

Figure 1 shows an apparatus for Total Internal Reflection Fluorescence Microscopy;

Figure 2 is a schematic showing the total internal reflection footprint walking with the displacement of the prism; and,

Figure 3 is a graph of db/dx against prism angle for a quartz/water base interface and a coupling angle of 68° .

Figure 1 illustrates an apparatus for Total Internal Reflection Fluorescence Microscopy having a light source 1 which generates a beam 2 which is incident on a prism 3 and is totally internally reflected at the base interface of the prism 3. A sample is positioned adjacent to a base interface of the prism 3 and the evanescent wave interacts with the sample, producing fluorescence. The fluorescence passes through an objective lens 4 and is directed towards a CCD camera 5 by a mirror 6, passing through a filter 7.

As shown in Figure 2, the light beam 2 is incident on the prism 3 at $\theta_i > \theta_c$ to the normal, is refracted as it enters the prism at θ_r to the normal and is incident on the base of the prism 3 at a coupling angle θ_c , forming a footprint at the base interface. For total internal reflection to

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occur, θ_c must be at least the critical angle for the base interface. As the prism moves dx in the lab frame position $3'$, the footprint walks db in the prism frame.

The magnitude of the differential dl/db may be derived as follows:- Sine rule $1 = r$ or $r \cos \theta_c = l \sin \theta_p$ (1) $\sin(90 - \theta_c) = \sin \theta_p$ Cosine rule $r^2 = b^2 + l^2 - 2bl \cos \theta_p$ (2) (1) in (2) $l^2 \sin^2 \theta_p = \cos^2 \theta_c (b^2 + l^2 - 2bl \cos \theta_p)$ quadratic in l $l^2 (\cos^2 \theta_c - \sin^2 \theta_p) - 2bl \cos \theta_p \cos^2 \theta_c + b^2 \cos^2 \theta_c = 0$ solution to which is

EMI7.1

expand contents of the square root

EMI7.2

take out $b^2 \cos^2 \theta_c$ common in the square root

EMI7.3

factorizing gives

EMI7.4

differentiating w. r. t. b

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EMI7.5

further factorization gives

EMI7.6

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recognising $\cos^2 \theta_p - 1 = -\sin^2 \theta_p$ and factorising

EMI8.1

recognising $1 - \cos^2 \theta = \sin^2 \theta$ and rooting the square $dl = \cos \theta (\cos \theta_p \cos \theta_c + \sin \theta_p \sin \theta_c) db (\cos^2 \theta_c - \sin^2 \theta_p)$ using trigonometric identity the differential simplifies to $dl = \cos \theta \cos (\theta_p + \theta_c) db (\cos^2 \theta_c - \sin^2 \theta_p)$

The magnitude of the differential dl/dx may be derived as follows:- Since ruled $dl = dx \sin(90 - \theta_p + \theta_i) \sin(180 - \theta_p - (90 - \theta_p + \theta_i))$ Simplifies to $dl \sin(90 - \theta_i) - dx \sin(90 - \theta_p + \theta_i) dl \cos \theta_i = dx \cos(\theta_p) dl = \cos \theta_p \cos \theta_i + \sin \theta_p \sin \theta_i dx \cos \theta_i$ $dl/dx = \cos \theta_p + \tan \theta_i \sin \theta_p$

Recognising that a translation of the prism dx in the positive x direction results in a displacement db of the footprint in the negative direction and by using the chain rule $-db = dl \times db/dx$ so $db = -(\cos \theta_p + \tan \theta_i \sin \theta_p) \times (\cos^2 \theta_c - \sin^2 \theta_p) dx \cos \theta_c \cos(\theta_p + \theta_c)$

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where $\theta_i = \sin^{-1}(n_p \sin(\theta_p - \theta_c))$ from Fresnel's equation, for light entering the prism from air, ($n_i = 1$).

For the footprint to remain stationary in the lab frame, it must walk in an equal and opposite direction in the prism frame to the prism's translation in the lab frame, i. e. $db/dx = -1$.

In total internal reflection microscopy, it is preferred that the coupling angle is greater than but close to the critical angle as this maximises the penetration of the evanescent wave into the sample medium, and it is generally preferable that θ_c is slightly above the critical angle because, although penetration of the evanescent wave is at a maximum at the critical angle, there will be a spread of angles within the beam and, to ensure total internal reflection of the entire beam, it is preferable to have θ_c slightly above the critical angle.

For a quartz/water interface at the base of the prism, where $n_p = 1.46$, the critical angle of the base interface is 66° . A preferred value for θ_c would be 68° . Figure 3 shows the degree of walking of the footprint with prism displacement as a function of the internal angle θ_p of the prism for $\theta_c = 68^\circ$. The prism angle required for $db/dx = -1$ is 68° and the light will be incident normal to the input surface of the prism, i. e. $\theta_i = 0$.

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Claims of WO03062897

CLAIMS1. A scanning apparatus comprising:- a light source for generating a light beam; and an angular prism coupled to a sample at a base interface; characterised in that the base angle θ_p of the prism satisfies the equations:- $(\cos^2 \theta_c - \sin^2 \theta_p) (\tan \theta_i \sin \theta_p + \cos \theta_p) = 1 \cos \theta_c \cos(\theta_p + \theta_c)$ and $n_i \sin \theta_i = n_p \sin(\theta_p - \theta_c)$ wherein θ_c is the coupling angle required for light incident at base interface of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.

2. A scanning apparatus according to claim 1, wherein $S_p = S_c$ and $\theta_i = 0$.
3. A total internal reflection microscopy apparatus according to claim 1 or 2, including means for detecting interaction of the sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.
4. A total internal reflection microscopy apparatus according to claim 3, wherein

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$S_c \sin^{-1}(n_s/n_p)$ and wherein n_s is the refractive index of the sample medium.

5. A scanning method comprising the steps of:- generating a light beam; providing an angular prism in the path of the light beam, the prism being coupled to a sample at a base interface; and moving the prism and sample relative to the light beam; characterised in that the base angle B_p of the prism satisfies the equations: $-(\cos^2 \theta_c - \sin^2 \theta_p) (\tan \theta_i \sin \theta_p + \cos \theta_c) \cos(\theta_p \pm \theta_c)$ and $n_i \sin \theta_i = n_p \sin(\theta_p \pm \theta_c)$ wherein θ_c is the coupling angle required for light incident at the interface at the base of the prism, θ_i is the incident angle of the light beam on the prism, n_i is the refractive index of the medium at the interface where the light beam enters the prism and n_p is the refractive index of the prism.
6. A method of total internal reflection microscopy according to claim 5, further comprising the step of detecting interaction of a sample at the base of the prism with an evanescent wave formed by total internal reflection of the light beam at the base of the prism.

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